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Prof. Joshua Lederberg
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Dear Prof. Lederberg:

I received your note last week requesting information about phloxine as a vital stain and the origin of the eosin-methylene blue agar medium.

With regard to phloxine, the dye was first synthesized in 1875. It is a bromofluorescein in the xanthene series, that is closely related to eosin, but has two chlorine atoms in the phthalic acid residue in addition to the four bromines. The Colour Index (C.I.) number is 45410. A closely related dye is phloxine B, which has four chlorines. The chemical structure of phloxine and a general write-up of its properties are given on page 347 of CONN'S BIOLOGICAL STAINS (9th edit., 1977; in the new edition which I am writing, there will be a separate chapter on fluorescent dyes and probes). Phloxine is fluorescent, as is eosin, fluorescein, etc.

Although not as popular as eosin, phloxine has occasionally been used as a substitute for eosin Y in cytoplasmic staining, giving a redder color than eosin. For instance, it was touted by Frank Mallory many years ago in his eosin-methylene blue tissue stain (when good lots of eosin were then hard to find) and is referred to today as Mallory's phloxine-methylene blue stain. The method gives brilliant coloration of connective tissues and of intranuclear inclusions in yellow fever (Emmel and Cowdry's LABORATORY TECHNIQUE IN BIOLOGY AND MEDICINE, 1964, page 338). There are several other histological stains that use phloxine. For instance, see the attached pages from Gurr (ENCYCLOPAEDIA OF MICROSCOPIC STAINS, 1960, pages 324-325).

You asked particularly about the use of phloxine as a vital stain. Although eosin has been employed as a fluorescent vital stain, phloxine does not seem to have been used this way. The closest use to vital staining that I could find for phloxine is in differentiating functional from abortive seeds, in combination with methyl green (Owczarzak, Stain Tech. 27:249, 1952) and in distinguishing two classes of virus inclusions in fresh plant tissues, in combination with trypan blue (McWhorter, Stain Tech. 11:107, 1936; 16:143, 1941).

There has been little use of phloxine in modern cell and molecular biology. Attached are three references with abstracts from my on-line computer search of Medline that relate to phloxine. The paper by de Weille et al. (1992) discusses the binding of fluorescein derivatives, including phloxine, to mnucleotide-binding sites on transport ATPases. A second article by Iwamoto et al. (1989) compares the relative photodynamic activity of phloxine and other food additive dyes. The third paper by Odds and Merson-Davies (1989) refers to the use of phloxine B agar to study Candida colony variations. A few other references that I picked up did not seem worth mentioning.

For discussions of vital staining, see the chapters by Foot and by Doan and Ralph (MCCLUNG'S HANDBOOK OF MICROSCOPICAL TECHNIQUE, 3rd Edit., Revised, 1950). There is an interesting chapter on dye diffusion into cells by Chambers and Chambers (EXPLORATIONS INTO THE NATURE OF THE LIVING CELL, 1961). Dye permeability into cells is treated by Jacobs in the book edited by Cowdry (GENERAL CYTOLOGY, 1924, see pages 135-139). Two other references that are valuable for reviews of the early literature on vital staining are by Conn and Cunningham (Stain Tech., 7:81, 1932) and Drawert (VITALFÄRBUNG UND VITALFLUOROCHROMIERUNG PFLANZLICHER ZELLEN UND GEWEBE. Protoplasmatologia, Vol. 2, Part D, No. 3, Springer Verlag, 1968).

More recent reviews of vital and supravital fluorochroming are given by Kasten (CELL STRUCTURE AND FUNCTION BY MICROSPECTROFLUOROMETRY, Edited by E. Kohen and J. Hirschberg, 1989, see pages 21-24; FLUORESCENT AND LUMINESCENT PROBES FOR BIOLOGICAL ACTIVITY, Edited by W. T. Mason, 1993, see pages 15-17). Vital fluorochroming in fluorescence microscopy and flow cytometry are discussed in various chapters of the books by Wang and Taylor (FLUORESCENCE MICROSCOPY OF LIVING CELLS IN CULTURE, Part A, 1989), Taylor and Wang (FLUORESCENCE MICROSCOPY OF LIVING CELLS IN CULTURE, Part B, 1989), and Darzynkiewicz and Crissman (FLOW CYTOMETRY, 1990).

Now for your second question on the eosin-methylene blue agar medium. I don't know where you excerpted the description that you sent me, but Levine's work was published in 1918, not 1917. Also, it should read Holt-Harris and Teague - not Holt, Harris and Teague. The basic medium originated with them in an article published in J. Infectious Diseases (18:596-600, 1916). The method was valuable in distinguishing E. coli (or B. coli as it was known then) from the typhoid bacillus. The rationale was exploited further by Teague and Harris, using eosin and Bismarck brown, to create a differential culture medium for the cholera vibrio (J. Infectious Diseases (18:601-605, 1916). Other papers were published during this period by Teague and associates where eosin-methylene blue agar, eosin-brilliant green agar, and the older Endo agar were compared for the isolation of typhoid bacilli (cf., J. Inf. Dis. 18:647, 1916; 18:653, 1916).

Although the eosin-methylene blue agar medium clearly originated with Holt-Harris and Teague in 1916, most people erroneously credit Levine. Levine came into the picture in 1918 with an article in J. Infectious Diseases (23:43-47, 1918). He acknowledged the prior work in this article and later ones. He modified slightly the eosin-methylene blue (EMB) agar medium of Holt-Harris and Teague to distinguish B. (E.) coli from B. (A.) aerogenes. The actual modification is ever so slight, that I wonder why Levine's name was retained alone by others for the name of the medium. In my opinion, this is a misnomer; the medium should be called "Holt-Harris, Teague, and Levine's EMB medium" or perhaps, "Levine's modification of Holt-Harris and Teague's eosin-methylene blue medium. A later article by Levine is also cited frequently when the EMB medium is referred to (Iowa State Engin. Exp. Stat., Bull. 62, 1921). For example, the DIFCO MANUAL cites this reference alone. The OXOID MANUAL OF CULTURE MEDIA, INGREDIENTS AND OTHER LABORATORY SERVICES gives the Levine references of both 1918 and 1921. Unfortunately, neither manual mentions the original work of Holt-Harris and Teague from 1916. The EMB medium was later used to identify Candida albicans. BERGEY'S MANUAL (Vol. 2, page 1545) refers to another long reference by Levine (Bull. Iowa State Agric. Coll.7:1-72, 1926).

The mistake I referred to above ("Holt, Harris and Teague") probably originated with Levine himself. In his 1918 paper, he refers correctly to Holt-Harris and Teague but in his 1920 article on page 38 (J. Inf. Dis. 27:31), he says, "Holt, Harris and Teague." I should be charitable in pointing out this error, since Levine was with the overseas American Expeditionary Forces in France at the time and may have been out of touch with library reference material.

Enclosed are photocopies of most of the material cited in the section of this letter on the eosin-methylene blue agar medium.

I hope that my response answers your questions. If there is anything further I can do, please let me know. I will naturally be interested to know more about your use of this information.

Sincerely,

Fred Kasten

Frederick H. Kasten, Ph.D.
Professor of Anatomy

Encl.